

## AMENDMENT

### Amendments to the Specification:

Following the abstract, please insert the attached Sequence Listing with subsequent page numbering thereafter.

Please replace paragraph [0016] with the following amended paragraph:

[0016] Polyanionic domains contain anionic amino acid residues, cationic amino acid residues, and non-ionic amino acid residues. Exemplary polyanionic domains have the formula  $[-(\text{Ala-Gly})_x\text{-Pro-Glu-Gly-}]_n$  (SEQ ID NO:1). The variable x is 0, 1, 2, 3, 4, 5, 6, 7, or 8, and the variable n is an integer from about 1 to 40. For example, a polyanionic domain includes a domain having the formula  $[-(\text{Ala-Gly})_x\text{-Pro-Glu-Gly-}]_n$ , where x is 3 and n is 16, and where x is 3 and n is 36. Additional exemplary polyanionic domains have the formula  $[-(\text{Ala-Gly})_x\text{-Glu-Gly-}]_n$  (SEQ ID NO:2). In this formula, the variable x is 0, 1, 2, 3, 4, 5, 6, 7, or 8, and the variable n is an integer from about 1 to 40. For example, polyanionic domain include a domain having the formula  $[-(\text{Ala-Gly})_x\text{-Glu-Gly-}]_n$ , where x is 4 and n is 16, 18 or 28, where x is 5 and n is 14, and where x is 6 and n is 14.

Please replace paragraph [0049] with the following amended paragraph:

[0016] Such polypeptides can be produced in *E. coli* in good yield and have been shown to adopt random coil or  $\beta$ -sheet structures (Krejchi et al., Science, 265:1427-1432 (1994) and McGrath et al., J. Am. Chem. Soc. 114:727-733 (1992). The periodic acidic side chains from the glutamic acid residues provide affinity to basic surfaces. The natural domain is a bacterial phosphotriesterase which catalyzes rapid hydrolysis of organophosphorus pesticides and nerve agents. The phosphotriesterase used here contains the modified amino acid sequence reported by Mulbry and coworkers, which is characterized by deletion of the 33 Amino-terminal amino acids of the native enzyme (from native sequence of *pseudomonas diminuta*) and replacement by the first 5 lac Z residues (Met-Ile-Thr-Asn-Ser-) (SEQ ID NO:3) followed by the enzyme residues (-Gly-Asp-Arg-), in order to achieve significant improvements in the



enzymatic activity in *E. coli* (Mulbry and Karns, *J. Bacteriol.* 171:6740-6746 (1989)). The activity of the enzyme can be easily determined using the pesticide paraoxon as substrate. Paraoxon can be enzymatically degraded to release a brightly colored phenolic product with a maximal absorption at 400 nm (Omburo et al., *J. Biol. Chem.* 267:13278-13283). The enzyme shows good stability and unusually high enzymatic activity, exhibiting a rate near the diffusion limit with substrate paraoxon (Caldwell et al., *Biochemistry*, 30:7438-7444 (1991)). The fusion proteins containing the repetitive polypeptides and phosphotriesterase adsorb to basic surfaces through the acidic artificial domain while the partner enzyme catalyzes hydrolysis of organophosphates.

Please replace paragraph [0051] with the following amended paragraph:

**[0051]**     *Recombinant DNA Construction.* Construction of the recombinant DNA encoding the fusion proteins is schematically shown in Figure 1. Plasmid pJK33 (obtained from Professor Frank Raushel of Texas A&M University) was digested with restriction enzymes BamH I and EcoR I. The resulting 1014 base pair subcloned *opd* gene encoding the phosphotriesterase was isolated from an agarose gel. The *opd* gene was ligated with a 21 base pair linker prepared by digestion of pUC18 with BamH I (SEQ ID NO:4) and EcoR I (SEQ ID NO:5). pET-3b was digested with BamH I to yield a linear fragment, which was purified on a 1% agarose gel. The linearized pET-3b fragment was combined with the linker modified *opd* gene (1:5 molar ratio) and incubated with T4 ligase at 15 °C overnight. The resulting recombinant DNA plasmid (designed pOL-1) was transformed into bacterial strain HB101. Colonies were screened for insertion and insert orientation by digestion with BamH I (supposed to yield 1035 base pair and 4639 base pair fragments) and EcoR I (supposed to yield 1524 and 4150 base pair fragments). pOL-1 was digested with Nde I and Pst I and the 2334 base pair DNA fragment was isolated by electrophoresis on a 1% agarose gel. pET-14b was digested with Nde I and Pst I and the 3399 base pair DNA fragment was purified as described above. The two gene fragments were incubated with T4 ligase, and the ligation product was used for transformation of *E. coli* strain BL21(DE3)pLysS. Transformants were selected and the DNA was verified by Ava I digestion (supposed to yield fragments of 486, 1254 and 3993 base pair). The resulting plasmid (pOL-2) was partially digested with BamH I



and the 5733 base pair fragment was incubated with DNA polymerase followed with ligation with T4 ligase to destroy the BamH I site on the 3' end. The plasmid was designated pOL-3. A family of fragments encoding the artificial domains was obtained from BamH I digestion of corresponding recombinant plasmids 21, 22, 28 and the appropriated fragment was inserted in the BamH I site of pOL-3. The resulting plasmids (pWD-X) contain a series of artificial coding sequences ligated in frame to the 5' end of the DNA encoding the modified phosphotriesterase of Mulbry and coworkers. Plasmids pWD-X were used to transform E. coli strain BL21(DE3) pLysS.